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Purification of a murine protein-tyrosine/threonine kinase that phosphorylates and activates the *Erk-1* gene product: Relationship to the fission yeast *byr1* gene product

(phorbol ester-activated threonine/tyrosine kinase)

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ABSTRACT **We report the purification to near homogeneity of a 45-kDa phorbol ester-stimulated protein kinase that phosphorylates and activates the *Erk-1* gene product. This kinase, which we provisionally denote MEK for MAPK/Erk kinase, phosphorylated kinase-inactive Erk-1 protein primarily on a tyrosine residue and, to a lesser extent, on a threonine. We extend our previous results and show that two forms of purified MEK activated the myelin basic protein kinase encoded by *Erk-1*. MEK was inactivated by the serine/threonine phosphatase 2A but not by the protein-tyrosine phosphatase 1B. Sequence analysis of peptides generated by trypsin digestion of MEK revealed similarity to the proteins encoded by the *Schizosaccharomyces pombe byr1* and *Saccharomyces cerevisiae STE7* genes. These data are discussed with regard to a possible signal transduction mechanism.**

Although control of enzymatic activity by reversible phosphorylation has been a well-established paradigm for several years (1, 2), the regulation of protein kinases involved in mitogenic signal transduction is only beginning to be clarified. The events leading to the phosphorylation of the ribosomal protein S6 have helped to define one model signal transduction pathway (for review, see ref. 3). The activity of the serine-specific S6 kinase pp90^{RSK} is positively regulated by the mitogen-activated protein (MAP)/myelin basic protein (MBP) kinase(s) encoded by the *Erk* (extracellular-signal regulated kinase) genes (4). The MAP/MBP kinase, which phosphorylates serine and threonine residues, is in turn regulated by threonine and tyrosine phosphorylation (5–9). Tyrosine phosphorylation of MAP/MBP kinases initially suggested that they may be direct targets for peptide growth factor protein-tyrosine kinases, but recent evidence indicates there may be at least one intervening protein kinase that mediates the growth factor receptor-induced activation of these enzymes (10–12). The molecular nature of the proteins leading to the phosphorylation of MAP/MBP kinases is still unclear, however, as other reports suggest an activation factor that is not a protein kinase (13, 14).

In this report we describe the purification and characterization of a phorbol ester-stimulated protein-tyrosine/threonine kinase that phosphorylates and activates the MBP kinase encoded by the *Erk-1* gene. This protein kinase is apparently regulated by serine/threonine phosphorylation. Peptide microsequence information shows that this enzyme, which we call MAPK/Erk kinase (MEK), shows sequence similarity to proteins encoded by the *Schizosaccharomyces pombe* (*Sc. pombe*) gene *byr1* and the *Saccharomyces cerevisiae* (*Sa. cerevisiae*) gene *STE7*.

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MATERIALS AND METHODS

Cell Culture. T-cell hybridomas (BY155.16.CD2-18) (15) were grown in Dulbecco's modified Eagle's medium/10% (vol/vol) heat-inactivated calf serum/0.03% glutamine/10 mM Hepes (pH 7.1) to a density of 10^6 cells per ml. Batches (6 liters) of cells were preincubated with 25 mM NaF for 60 min, centrifuged at 300 × *g* for 10 min, resuspended in 160 ml of medium, and stimulated with phorbol 12-myristate 13-acetate (PMA; 100 ng/ml) for 5 min. Cell pellets were quick-frozen in liquid nitrogen and stored at −70°C.

Kinase Assays. Samples (5 μl) of column fractions were assayed as described (16) for kinase activity by incubation with 1 μg of the kinase-inactive mutant glutathione *S*-transferase (GST)–Erk-1(K63M) in 30 μl of 50 mM Tris·HCl, pH 8.0/3 mM magnesium acetate/50 μM ATP (5 μCi of [γ-^{32}P]ATP; 1 Ci = 37 GBq)/5 mM dithiothreitol/ovalbumin (0.1 mg/ml) for 10 min. All reactions except those from the Fast Flow S column were terminated by the addition of 5× Laemmli SDS loading buffer (17). Proteins were separated on 8% polyacrylamide gels, and ^{32}P was detected by autoradiography. The appropriate bands were excised from the dried gels and incorporated ^{32}P was measured by liquid scintillation. To reduce background, the products of the protein kinase reactions from the Fast Flow S column were terminated by incubation with 100 μl of 20% (wt/vol) glutathione-agarose (Sigma) in ice-cold phosphate-buffered saline for 15 min on ice and washed with 1 ml of buffer containing 1% Nonidet P-40/0.5% sodium deoxycholate/100 mM NaCl/10 mM Tris·HCl, pH 7.3/10 mM EDTA. The beads were resuspended in 25 μl of 1× Laemmli SDS loading buffer and proteins were separated by SDS/PAGE. The MBP kinase assays were carried out as described (18).

Activation Time Course. Samples (1.5 ml) of T-cell hybridomas (10^6 cells per ml) were removed at the designated times after stimulation with phorbol ester (100 ng/ml) and pelleted briefly before freezing with liquid nitrogen. Cell pellets were lysed in 0.5 ml of lysis buffer consisting of column buffer A [25 mM Mes·KOH, pH 6.5/10 mM NaF/1 mM EDTA/5 mM EGTA/5% (vol/vol) glycerol/15 mM 2-mercaptoethanol/0.1% Triton X-100], supplemented with additional Triton X-100 (0.5%, final concentration) and protease inhibitors [1 mM phenylmethylsulfonyl fluoride/leupeptin (10 μg/ml)/pepstatin A (10 μg/ml)]. Pellets were homogenized with a Dounce homogenizer and centrifuged for 30 min at 100,000 × *g*. Supernatants were assayed in triplicate for GST–Erk-1-(K63M) (MEK) activity and for MBP kinase activity as described (18). In addition, the MBP kinase assay conditions used here contained 6 μM protein kinase A inhibitor peptide (Sigma).

Abbreviations: GST, glutathione *S*-transferase; MAP, mitogen-activated protein; MBP, myelin basic protein; MEK, MAPK/Erk kinase; PMA, phorbol 12-myristate 13-acetate.

Chromatography. Frozen pellets from 30 liters of stimulated T-cell hybridomas were homogenized in 100 ml of lysis buffer containing 50 mM NaCl with a Tissumizer (Tekmar, Cincinnati) at 60% speed for three 45-sec periods. After a 30-min 100,000 × *g* centrifugation, the extract was filtered through glass wool and then adsorbed to 40 g of Whatman DE52 DEAE-cellulose for 30 min with stirring. The column supernatant was loaded onto a 20-ml Fast Flow S column (Pharmacia) and a 200-ml 0–0.5 M NaCl gradient was developed at a flow rate of 2.5 ml/min.

The two Fast Flow S peaks of MEK activity were combined and dialyzed against buffer B (20 mM Tris·HCl, pH 7.5/50 mM glycerol 2-phosphate/0.1 mM EDTA/5 mM NaF/15 mM 2-mercaptoethanol) before loading onto a 40-ml Hiload HPQ column (Pharmacia). This column was washed with buffer B and proteins were eluted with a 200-ml 0–0.5 M NaCl gradient at 5 ml/min. Two peaks of MEK activity (pools A and B) were pooled separately and each peak was individually purified further by sequential chromatography beginning with a 20-ml heparin-Sepharose (Pharmacia) column. This column elution gradient was developed with a 200-ml 0–0.5 M NaCl gradient in buffer B at 0.8 ml/min.

The enzymatic activity peak from the heparin-Sepharose column was dialyzed against buffer C (10 mM potassium phosphate, pH 7.1/1 mM EDTA/1 mM EGTA/5 mM $MgCl_2$/0.1 mM Na_3VO_4/2 mM dithiothreitol/0.1% Triton X-100/5% glycerol) and then loaded onto a 1-ml Mono S HR5/5 column (Pharmacia). The breakthrough fractions were dialyzed against buffer A and reloaded onto the Mono S column equilibrated with buffer A. Bound proteins were eluted with a 40-ml 0–0.5 M NaCl gradient at 1 ml/min and 1-ml fractions were collected. The purity of the proteins in Mono S pools A and B was determined by SDS/PAGE followed by visualization with the Silver Stain Plus kit (Bio-Rad).

Phosphoamino Acid Analysis. Phosphoamino acid analysis was performed as described (18) with the following modifications: 2.5 ng of purified Mono S pools A and B were used to phosphorylate GST–Erk-1(K63M) as described above for 20 min or were allowed to autophosphorylate in 50 mM Tris·HCl, 8.0/3 mM magnesium acetate/20 μCi of [γ-^{32}P]ATP/5 mM dithiothreitol for 20 min.

Peptide Microsequencing. A 45- to 46-kDa band was purified from 36 liters of NaF/PMA-stimulated hybridomas using a modified purification protocol in which the Hiload HPQ peaks were recombined and further chromatographed as described above. The resulting Mono S fractions containing the protein kinase were resolved on a 10% preparative polyacrylamide gel, electrophoretically transferred to nitrocellulose, and the 45- to 46-kDa band was *in situ*-digested with trypsin (19). The tryptic peptides were separated by HPLC, and six peptides were sequenced using an automated peptide sequencer as described (20).

RESULTS

Activation of MEK Activity. To determine the kinetics of phorbol ester-induced Erk kinases in T-cell hybridomas, we assayed lysates for the ability to phosphorylate GST–Erk-1(K63M) in cells stimulated for 0.5, 1, 3, 5, 10, 15, 30, or 180 min after PMA addition. To eliminate background due to autophosphorylation of bacterially produced Erk-1 (14, 18, 21), the kinase-inactive Erk-1 mutant GST–Erk-1(K63M) was used as substrate in these assays, as described (16). Although a poor substrate when denatured (data not shown), native GST–Erk-1(K63M) serves readily as a phosphate acceptor molecule for a kinase activity that is stimulated about 8-fold upon PMA addition (Fig. 1). This increased activity was 42% of maximum within 30 sec and peaked in 3 min before declining linearly to 29% of maximum after 3 h. The activation of PMA-stimulated MBP kinases lagged slightly behind

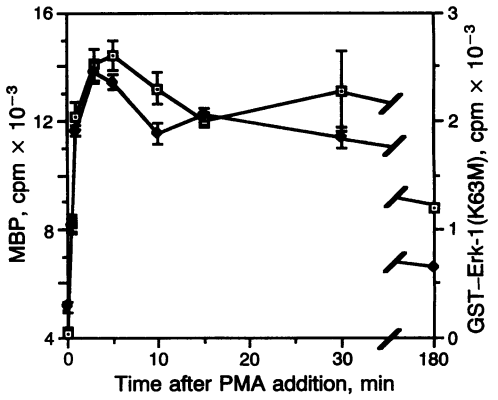

FIG. 1. Time course of MEK and MBP kinase activation. T-cell hybridomas were stimulated with PMA (100 ng/ml) and samples were removed after 0.5, 1, 3, 5, 10, 15, 30, or 180 min. Cell lysates were assayed in triplicate for MEK (◆) and MBP kinase activity (□).

the activation of MEK under these conditions. MBP kinases reached peak activity within 5 min after stimulation and decreased with kinetics similar to that of MEK activity.

MEK Purification. Since treatment with NaF, an inhibitor of serine/threonine phosphatases, increased the basal level of MEK activity, cells were pretreated with NaF for 60 min prior to the addition of PMA. When cells were homogenized in buffer A, MEK activity was present in both the cytosolic and particulate fractions of a high-speed centrifugation (data not shown) but was solubilized in 0.5% Triton X-100; therefore, this amount of detergent was present in the lysis buffer. The kinase activity bound very weakly to a DEAE-cellulose matrix under buffer A conditions and remained in the supernatant after batch loading the 100,000 × *g* supernatant onto DEAE-cellulose in the presence of 50 mM NaCl. The resulting DEAE-cellulose supernatant had 5-fold more total activity than the lysate applied to the matrix (data not shown). Two peaks of MEK activity were resolved by chromatography on the Fast Flow S column (70–115 mM NaCl and 170–235 mM NaCl; Fig. 2*A*). These peaks were combined and, when fractionated on a Hiload HPQ column, two peaks were again resolved (peak A, wash fractions; peak B, 15–105 mM NaCl) (Fig. 2*B*). Hiload HPQ column peak A was eluted during the wash step after the majority of unbound proteins had flowed through the column as monitored by UV absorbance. In this regard, the treatment of this column differs from that described in ref. 16, where the total flow-through and wash fractions were pooled but not used in the analysis of Erk-1 phosphorylation. Each Hiload HPQ peak was pooled separately and further purified using heparin-Sepharose and Mono S columns. Activity profiles for pool A are representative for both pools (Fig. 2 *C* and *D*) since they chromatographed similarly on these columns. Although a fraction of the total MEK activity in either pool A or pool B did not bind to heparin-Sepharose, the major peak of activity from both eluted between 85 and 165 mM NaCl. Additional purification was achieved by taking advantage of the fact that MEK activity from either pool A or B did not adsorb to the cation-exchange Mono S column under buffer C conditions (pH 7.1). MEK activity did, however, bind to this matrix under buffer A conditions (pH 6.5) and was eluted between 125 and 225 mM NaCl. The two MEK activities were purified >20,000- and 40,000-fold, respectively, from 30 liters of NaF/PMA-stimulated T-cell hybridomas, with a combined overall yield of 32% of the MEK activity present in the DEAE supernatant. Separation by SDS/PAGE and quantitation by silver stain analysis with bovine serum albumin standards showed that this purification protocol yielded ≈33 pmol of a nearly homogenous 45-kDa protein in Mono S pool A (Fig. 3*A*, lane 1) with a specific activity of 31 nmol per min per mg.

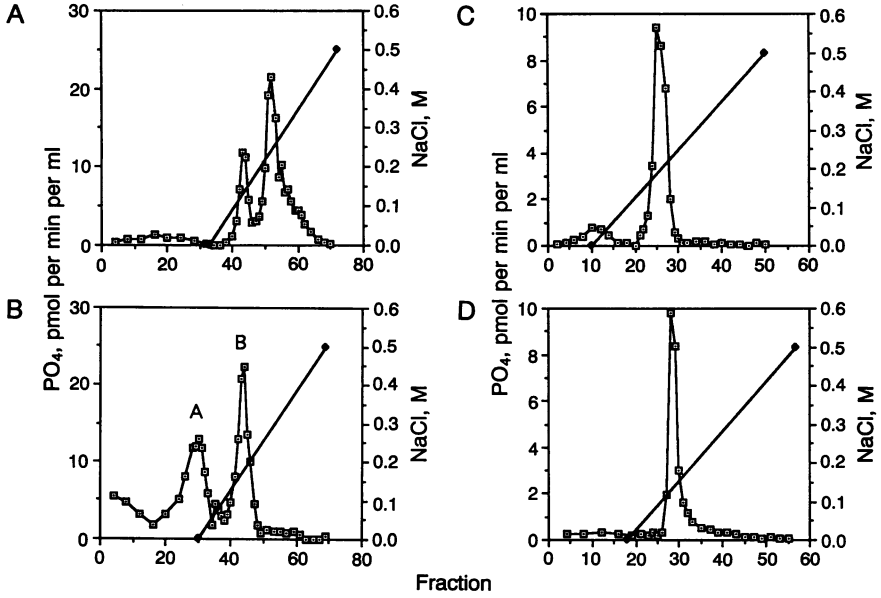

FIG. 2. Purification of MEK activity from PMA-stimulated cells. By using the kinase-inactive mutant GST–Erk-1(K63M) as substrate, MEK activity was measured in fractions from the following columns; Fast Flow S Sepharose (*A*), Hiload high performance Q (HPQ) Sepharose (*B*), heparin-Sepharose (*C*), and Mono S (*D*). The Fast Flow S peaks (*A*) were combined and loaded onto the Hiload HPQ column (*B*), which separated MEK activity into two peaks, peaks A and B. These peaks were individually purified further. (*C* and *D*) Only pool A profiles are shown, since pools A and B chromatographed similarly on these columns.

The 45- to 46-kDa band in Mono S pool B represented ≈50% of the total protein and contained ≈43 pmol (Fig. 3*A*, lane 2), with a specific activity of 68 nmol per min per mg. The pool B band was less sharp than the pool A band, which may suggest heterogeneity due to post-translational modification. These 45- to 46-kDa proteins were eluted with the peak of GST–Erk-1(K63M) activity from both the heparin-Sepharose and Mono S columns (data not shown). In addition, these two bands correlated with two phosphorylated bands when samples of pools A and B were incubated under autophosphorylation conditions (data not shown).

To determine whether the MEK activity in Mono S pools A and B is regulated by phosphorylation, we treated a sample of each preparation with the serine/threonine phosphatase 2A or the protein-tyrosine phosphatase 1B. Phosphatase 2A treatment reduced pool A activity by 79% and pool B activity by 89%, whereas protein-tyrosine phosphatase 1B had no effect on the activity of pool A or B (data not shown).

Phosphoamino Acid Analysis. Phosphoamino acid analysis showed that the 45- to 46-kDa bands in Mono S pools A and B autophosphorylated predominately on threonine but also on serine and tyrosine residues (Fig. 3*B*, lanes 2 and 4). Both preparations, however, phosphorylated GST–Erk-1(K63M) on tyrosine and to a lesser extent on threonine (Fig. 3*B*, lanes 1 and 3). These sites of phosphorylation by MEK are required for MAP/MBP kinase activation, as documented (16, 22, 23).

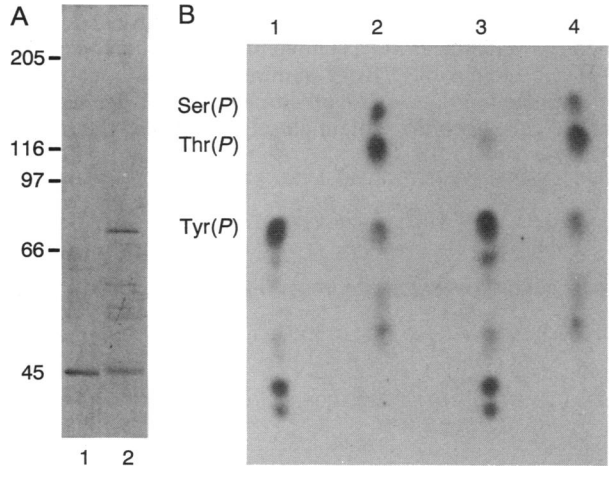

FIG. 3. Silver stain and phosphoamino acid analyses of purified MEK. (*A*) Silver stain. Samples of Mono S pools A (lane 1) and B (lane 2) were analyzed by SDS/PAGE and silver-stained. Molecular masses in kDa are shown. (*B*) Phosphoamino acid analysis. GST–Erk-1(K63M) phosphorylated by Mono S pools A (lane 1) and B (lane 3) and autophosphorylated 45-kDa band from Mono S pools A (lane 2) and B (lane 4) are shown.

Activation of Recombinant Erk-1 by Peaks A and B. Mono S pools A and B were tested for the ability to activate recombinant murine Erk-1 *in vitro*. As shown in Fig. 4, preincubation of pool A or pool B with bacterially produced GST–Erk-1 and MgATP increased the MBP kinase activity of the recombinant Erk-1 fusion protein. The rate of GST–Erk-1 activation plateaued between 15 and 30 min for pools A and B, and after 60 min, the MBP kinase activity was increased 16- and 33-fold over basal levels for pools A and B, respectively. Similar results were obtained with the partially purified enzyme as described (16).

Sequence Comparison with *Sc. pombe byr1*. To obtain enough purified protein to get peptide sequence information, we started with 36 liters of NaF/PMA-stimulated T-cell hybridomas and modified the MEK purification protocol described above by recombining the two MEK activity peaks separated on both the Fast Flow S and Hiload HPQ columns before further chromatography on heparin-Sepharose and Mono S columns. This modified protocol yielded ≈93 pmol of a 45- to 46-kDa diffuse band. Tryptic peptides of this band were HPLC-purified and six peptides were sequenced using an ABI 477A protein sequencer (Table 1). Four of the six peptides aligned closely with the amino acid sequences of the

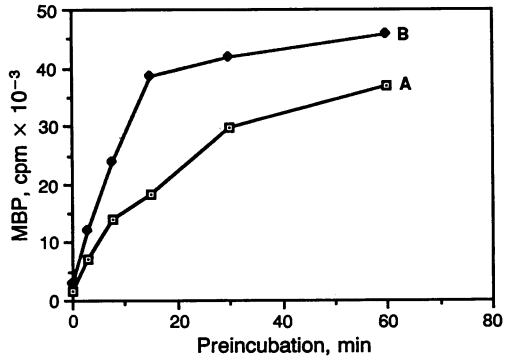

FIG. 4. Recombinant GST–Erk-1 activation by MEK. GST–Erk-1 (1.5 μg) was preincubated with 4.5 ng of Mono S pool A (◆) or B (□) and MgATP for the designated times before assaying MBP kinase activity in triplicate.

Table 1. MEK tryptic peptide sequences

Peptide	Sequence	Byr1 relationship
1	LCDFGVSGQLIDSMANSFVGTR	Yes
2	ELQVLHEXNSPYIVGFYGAFYSD	Yes
3	ISELGAGNGGVVFK	Yes
4	SYMSPER	Yes
5	KKPTPIQLNPAPDGSAV	Possible
6	RSDAEEVDFAGQLXSTIXLNQP	?

Tryptic peptide sequences from purified MEK. A modified purification protocol, in which the Hiload HPQ pools A and B were combined, yielded a diffuse 45- to 46-kDa band. This band was trypsin-digested, and six HPLC purified peptides were sequenced with an automated peptide sequencer. An X represents an amino acid whose identity could not be determined. These unidentified amino acids could be either cysteine or modified amino acids such as phosphoamino acids. Peptide sequencing was carried out in the Harvard Microchemistry Facility under the direction of William Lane.

Sc. pombe byr1 gene product (24) (Fig. 5) sharing 50–86% identity and 57–86% similarity within the canonical kinase catalytic domains identified by Hanks *et al.* (26). In addition, these peptides shared comparable similarities with the amino acid sequence of the *Sa. cerevisiae* kinase STE7 (27) (data not shown).

DISCUSSION

We report here the purification and partial characterization of phorbol ester-stimulated murine protein kinase activities that can phosphorylate and activate recombinant Erk-1 16- and 33-fold *in vitro*. These activities, as shown here and in ref. 16, are evidently protein kinases, which we have named MEK. In total cell lysates, the activity peaks within 3 min of PMA addition and precedes the peak of MBP kinase activity, suggesting that MEK may activate MBP kinase *in vivo*. These two purified 45- to 46-kDa proteins (Fig. 3*A*) may represent a single gene product that is differentially modified or may be two closely related members of a tyrosyl/threonyl kinase family. The pools designated A and B share several common characteristics such as electrophoretic mobility, specificity of autophosphorylated phosphoamino acids, threonyl and tyrosyl substrate specificity, and the ability to phosphorylate the threonine and tyrosine residues on murine Erk-1 that have been identified as regulatory phosphorylation sites (23, 28). Furthermore, both preparations can also be inactivated with the serine/threonine-specific phosphatase 2A, whereas the tyrosine-specific phosphatase 1B has no effect. Pool A differs from pool B, however, with respect to specific activities, chromatographic behavior on Fast Flow S and Hiload HPQ columns, and kinetics of recombinant Erk-1 activation.

Sequence analysis of tryptic peptides derived from the purified 45- to 46-kDa protein demonstrates that MEK has a high degree of sequence similarity to the yeast *byr1* and *STE7* gene products, which are allegedly protein kinases (24, 27). Four of the six tryptic peptide sequences can be closely matched to sequences in *byr1* (Fig. 5); however, peptide 5 is only distantly related to byr1 or, in data not shown, to STE7. Peptide 6 does not appear to be related to byr1 or STE7 and thus indicates MEK may represent a unique member of this putative gene family. In this regard, it should be noted that the predicated *byr1* gene product is ≈38 kDa, whereas the STE7 product is expected to be ≈55 kDa. This significant difference in molecular mass suggests that MEK may not subsume all the functions of either *byr1* or *STE7*.

The discovery that a single enzyme is apparently capable of activating the MBP (MAP) kinase encoded by the *Erk-1* gene *in vitro* sheds light on the hierarchical mechanism of this signal transduction pathway. MAP (Erk) kinase has been proposed as an integration point in mitogenic signal transduction, since its activation requires the phosphorylation of both tyrosine and threonine residues (5). However, the identification and purification of MEK, a tyrosyl- and threonyl-specific kinase capable of activating recombinant Erk-1 *in vitro*, suggests that *in vivo* activation of MAP kinase(s) may instead proceed via the activation of MEK or a family of closely related tyrosyl/threonyl kinases. The possibility exists, however, that although the 45-kDa protein kinase in Mono S pool A was purified to near homogeneity, additional undetected protein kinases may contribute to the activation. This question will best be addressed after the cloning and expression of a MEK cDNA.

Other laboratories have reported MAP/MBP kinase activation. Ahn *et al.* (13) initially described two epidermal growth factor-stimulated activities that could activate inactive MBP kinases from unstimulated cells. Because these activities could not phosphorylate any tested exogenous substrates and the MBP kinases used have the capacity to autophosphorylate *in vitro* (14, 18, 21), it was not possible to classify these activities as kinases. Gómez and Cohen (29) described two protein kinase activities that catalyzed the partial reactivation and phosphorylation of phosphatase-treated MAP/MBP kinase on threonine, serine, and tyrosine. These two MAP kinase kinase activities were in turn inactivated by treatment with protein phosphatase 2A. More recently, Matsuda *et al.* (11) have purified to homogeneity a 45-kDa protein kinase from mature *Xenopus* oocytes that could phosphorylate and activate inactive *Xenopus* MAP kinase. This *Xenopus* protein closely resembles the purified

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1   MFKRRRNPKGLVLNPNASVKSSDNDHKEELINNQKSFESNVEAFMEQCAHMNRRPAWISDLDNSSLEVVRHLGEGNGGAVSLVKHRNIFMARKTVYVGSD  100
          :.|..: |||.:....                                                :..||.||||.|
    .....KKPTPIQLNPAPDGSAV.....................................................ISELGAGNGGVVFK.................

101 SKLQKQILRELGVLHHCRSPYIVGFYGAFQYKNNISLCMEYMDCGSLDAILREGGPIPLDILGKIINSMVKGLIYLYNVLHIIHRDLKPSNVVVNSRGEI  200
              ||.|||.|.|||||||||||| |.:
    .........ELQVLHECNSPYIVGFYGAF.YSD.....................................................................

201 KLCDFGVSGELVNSVAQTFVGTSTYMSPERIRGGKYTVKSDIWSLGISIIELATQELPWSFSNIDDSIGILDLLHCIVQEEPPRLPSSFPEDLRLFVDAC  300
     ||||||||:|::|:|..||||..||||||
    .LCDFGVSGQLIDSMANSFVGTRSYMSPER.....................................................................

301 LHKDPTLRASPQQLCAMPYFQQALMINVDLASWASNFRSS  341

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FIG. 5. Amino acid sequence alignment of MEK tryptic peptides and *Sc. pombe* byr1. MEK tryptic peptides were aligned with the *Sc. pombe* byr1 amino acid sequence using the GCG GAP program. Vertical lines indicate identity, and colons and periods indicate conserved changes according to the described programs (25).

kinase described in this communication in terms of size, chromatographic behavior, and sensitivity to phosphatase 2A. After this work was completed, L'Allemain *et al.* (12) also reported that phorbol ester or mammalian growth factor induces a p42/MAP kinase kinase activity that phosphorylates tyrosine and threonine residues of the protein produced in bacteria.

We have shown by peptide sequencing that at least one MAP/MBP kinase "activator" is a protein kinase and is highly similar to the *Sc. pombe byr1* and *Sa. cerevisiae STE7* gene products. Therefore, MEK may represent a mammalian counterpart of these yeast kinases. Results from recent experiments using a dominant negative $p21^{ras}$ mutant or an overexpressed ras growth-associated protein suggest a role for $p21^{ras}$ in growth factor- and protein kinase C-initiated MAP kinase activation (30–33). In this regard, it is notable that *Sc. pombe byr1* has been shown to be involved in mediating Ras signal transduction during yeast mating (34). In *Sc. pombe*, the gene for another putative protein-serine/threonine kinase, *byr2*, which also lies downstream of *ras*, has been identified (35). Overexpression of *byr1* overcomes the sporulation defects of diploid yeast lacking *byr2*, thus suggesting the actions of *byr2* lie between *ras* and *byr1* (35). Yeast mating involves a number of events, such as response to peptide factors and transcriptional regulation, that may be in part analogous to biochemical pathways that require the activation of MEK in animal cells. Thus, the evidence that MAP/MBP kinase activation may be downstream of *ras* in animal cells and the strong sequence similarity between *Sc. pombe byr1* and MEK suggest the presence of an unidentified mammalian homolog to *byr2*, which mediates $p21^{ras}$-induced activation of MEK.

We are grateful to Alessandro Alessandrini for a gift of the GST–Erk-1(K63M) fusion protein necessary for the purification described here and for many helpful discussions. We also thank Lucy Rodrigues for a constant supply of T cells. William Lane in the Harvard Microchemistry Facility provided indispensable encouragement and advice on peptide sequencing and analysis. We also thank David L. Brautigan for a gift of protein phosphatase 2A. This research was supported by National Institutes of Health Grant CA42580 (R.L.E.) and National Institutes of Health Genetics Training Grant GM07620-14 (C.M.C.). R.L.E. is an American Cancer Society Professor of Cellular and Developmental Biology.

Note Added in Proof. After the submission of this manuscript, Segar *et al.* (36) reported the purification of an epidermal growth factor-stimulated MAP kinase activator.

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